

# **Quantifying Trophic and Demographic Rates of Plankton-Rich Layers in East Sound, Orcas Island, Washington**

Susanne Menden-Deuer  
Professor in Residence  
University of Rhode Island  
Graduate School of Oceanography  
South Ferry Road, Bay Campus  
Narragansett, RI 02882  
phone: (401) 874-6608 fax (401)-874-6728 email: [smenden@gso.uri.edu](mailto:smenden@gso.uri.edu)

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## **LONG-TERM GOALS**

The goal of this research is to develop a mechanistic understanding and predictive capability of how biological processes affect plankton patch formation, persistence and decline. This goal will be addressed by concurrent characterization of the physical, chemical and biological parameters associated with patch occurrence and dissipation with biologically meaningful resolution (meters and days).

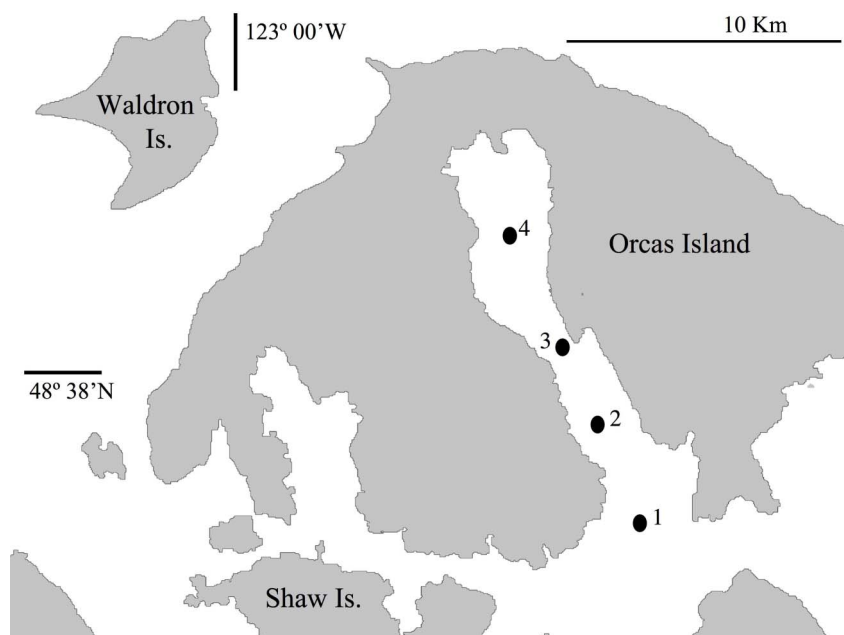
## **OBJECTIVES**

The objective of the funded work is to quantify the contribution of ecological processes to patch formation in the coastal ocean. These objectives will be addressed by simultaneously quantify (1) spatial and temporal characteristics of large plankton patches, (2) the physical and chemical conditions these patches occur in and (3) the plankton population dynamics of the dominant layer forming species through simultaneous measurements of the biological growth and mortality rates.

## **APPROACH**

The methodological approach of this research is to combine small boat surveys, during which we characterize field conditions and capture samples, with laboratory measurements of plankton growth and grazing rates as well as quantification of nutrient, biomass and Chl *a* concentrations. This research utilizes methods established during my previous work in the same area (Menden-Deuer, in press). A major focus of this effort is to link ecological rates and taxonomic composition, parameters that require considerable resources, time and expertise to acquire, with parameters that can more easily be measured automatically and thus at higher resolution.

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*Figure 1. Station locations in East Sound, Orcas Island, WA.*

**Small boat surveys, East Sound, Orcas Island** East Sound is a temperate fjord within the San Juan Archipelago in the Northeastern Pacific (N 48° 38.61', W 122° 52.75', Figure 1). The fjord has a north-south extent of approximately 9 km, an east-west width of 1 - 2 km and mean depth of 30 m. Circulation and exchange with the tidally well-mixed water outside is restricted by a partial sill at the southwestern terminus of the fjord. Previous, ONR funded work has established East Sound, Washington as a site of recurring plankton layer presences and provided great insight into the physical forcing mechanisms (Dekshenieks et al. 2001). The presence of distinct plankton layers was subsequently confirmed in a variety of coastal environments, highlighting that layers are a common rather than rare occurrences (McManus et al. 2005). From our land base, at the Shannon Point Marine Center, part of Western Washington University, East Sound is easily accessible by small boat within 30 - 45 minutes. Previous work has established that plankton layers in East Sound are continuous and coherent structures on a daily basis, but that significant changes in the composition occurs on the order of a few days (Menden-Deuer, in press). Based on these findings, our small boat surveys are carried out on alternating days. A total of four stations are sampled on each day, three established stations within East Sound and the reference station outside the sound, where no layers have been observed. Layer presence is determined by profiling the water column with a SeaBird 19+ CTD (T, S, P,  $\sigma_t$ ) and mounted fluorometer (Wetlabs WetStar). Layers are sampled during the summer at intervals of 1-3 days for a total of 2-3 weeks, to relate short-term changes (between days) in layer characteristics to longer-term changes (between weeks). By conducting this work over several days, predictions of changes in plankton layer intensity will be comparable to subsequent, observed plankton rich layer (PRL) intensities. Water samples from within PRLs and surrounding waters will be collected with a 2L and 10L horizontally-mounted Niskin bottle.

**Biological quantities and rate measurements** At the laboratory, water samples from all stations and all depths are analyzed for extracted Chl *a* and nutrient (phosphate, nitrate and silicate) concentrations. Whole water samples are preserved with Lugol's iodine to a final concentration of 2% (Menden-Deuer

et al. 2001) for later taxonomic analysis. These quantities place the field and rate measurement data in a quantitative, biological context. To establish the rates of change of plankton three different methods are used, two independent methods to measure primary productivity and the dilution method to measure heterotrophic protists grazing rate. In combination, these methods quantify both the potential for increase as well as decrease in the total population. In the context of the other measurements outlined above, they provide a synoptic picture of the factors controlling plankton biomass: standing stock, available nutrients, grazer induced mortality and environmental stability.

The rate of change in phytoplankton biomass is measured using the radio-labeling method that was first developed by Steemann Nielsen (1952) to quantify photosynthesis. Since then, the method has been effectively applied to measure phototrophic processes, including phytoplankton growth rates, carbon to *Chl a* ratios (Welschmeyer & Lorenzen, 1984) and cellular carbon content (Putt & Stoecker 1989, Crawford & Stoecker 1996, Menden-Deuer & Lessard 2000). The radio-labeling technique exploits the fact that photosynthetic organisms incorporate inorganic CO<sub>2</sub> to generate their tissue and measurements with a scintillation counter are sensitive enough to detect <sup>14</sup>CO<sub>2</sub> within a single cell (e.g. Menden-Deuer & Lessard 2000). A known fraction of the total CO<sub>2</sub> is offered as a radio-labeled tracer (<sup>14</sup>CO<sub>2</sub>). The uptake rate of the tracer can then be used to calculate photosynthetic rates. Here this method will be applied in two variations:

First, standard primary productivity experiments are run under in-situ conditions by incubating radio-labeled samples in an incubator that is cooled with ambient seawater and exposed to ambient surface light. The light levels of the incubations span the range of light levels observed in the field. Light levels are adjusted using neutral density screen, resulting in typically 6 independent incubations, per station and depth, without replication. Samples are incubated for 2 hrs on a rotating wheel to ensure mixing of the contents. The experiments are terminated by filtering of the samples and acidification. Incorporation of radio-active isotope is measured using a standard scintillation counter. Measures of decays per minute are converted to *Chl a* and C specific production rates.

The second primary productivity experiment is conducted in a controlled light box, with positions that correspond to known light levels. This approach allows much greater replication, and in these experiments, 14 light levels are used to estimate the rate of photosynthetic activity and capacity. The advantage of this experimental approach is the much greater replication as well as estimation of photosynthetic potential. The disadvantage is that the incubation corresponds less to in-situ conditions than the method outlined above. To offset this disadvantage, we designed the light levels such, that they would span the range of intensities plankton would experience in summer in the coastal, temperate ocean. During incubation, samples are cooled to ambient water temperatures.

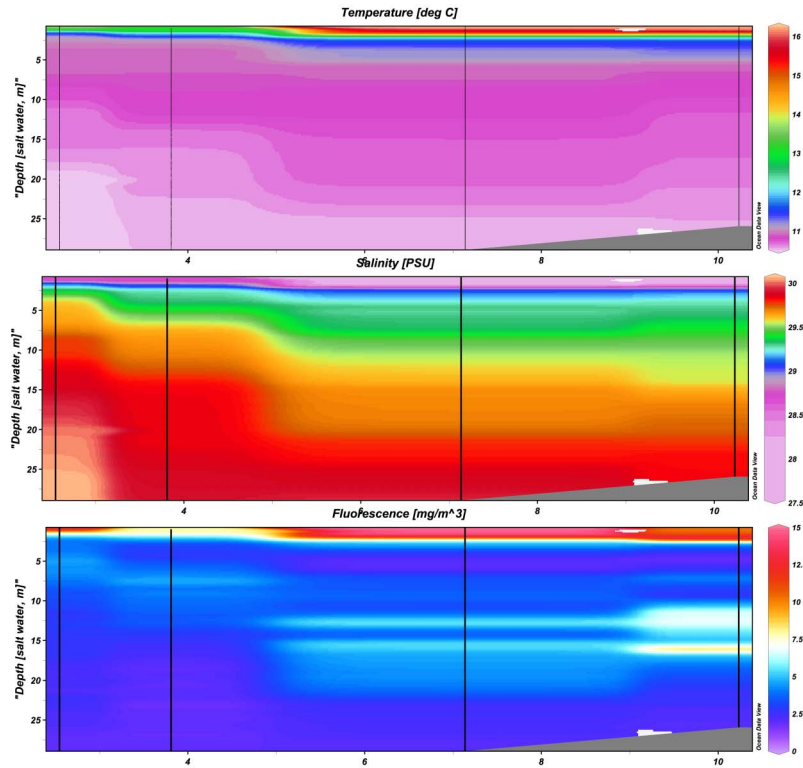
The dilution method (Landry and Hassett 1982) is used to complete the assessment of biological processes that alter plankton standing stock and productivity. Specifically, it was used to assess potential loss of phytoplankton due to grazing mortality and subsequent increases in zooplankton due to growth. The dilution experiments were conducted according to protocols established by Suzanne Strom (WWU) and her laboratory. Whole water samples were prescreened through a 200 µm mesh, so that larger zooplankton were eliminated from the experiments, to avoid grazing of copepods on the microzooplankton predators. Two dilution levels (5 and 100%) were run in triplicate, some experiments had an additional, nutrient addition treatment to avoid nutrient starvation of the primary producers. All samples were incubated for 24 hrs in an incubator, cooled with ambient seawater and exposed to ambient surface light levels. Light levels were adjusted to the sample depth through neutral density screen.

This work builds upon prior ONR funded work in East Sound conducted by, in alphabetical order: Alldredge, Cowles, Donaghay, Grünbaum, Holliday, McManus, Perry, and Zaneveld. This project was conducted simultaneously, and in collaboration with Tatiana Rynearson investigating how genetic diversity affects the development and persistence of plankton layers.

## **WORK COMPLETED**

The work completed so far has exceeded the originally proposed work in a number of aspects. During July and August 2007 my lab spent seven weeks at the Shannon Point Marine Center, Anacortes, Washington. During that time, we conducted 15 day cruises to East Sound, Orcas Island. On each of these day cruises, a total of four stations were visited, three within East Sound and one reference station outside the sound (Figure 1). At each station, a vertical profile of the physical properties of the water column as well as phytoplankton fluorescence were recorded with a SeaBird CTD 19+ at a vertical resolution of approximately 0.2 m. A hand-held light meter (Li-Cor, LI-1400, with an underwater spherical quantum sensor SPQA 3585) was used to acquire a light profile of the water column. Real time acquisition of fluorescence data allowed us to identify phytoplankton layer presence. At each station, two depths were sampled, with the sample volume depending on the planned laboratory experiments for that day. On all days, at all stations, water samples were collected for preserved whole water samples for taxonomic analysis, a size fractionated, triplicate Chl *a* analysis (5, 10 and 20  $\mu\text{m}$ ) as well as analysis of dissolved inorganic nutrients (phosphate, nitrates and silicates). In total, nearly 1100 Chl *a* samples were taken, 200 nutrient samples were analyzed for dissolved phosphate, nitrate and silicate concentrations as well as over 100 samples preserved for taxonomic identification of the major plankton species in the size range of 5 to 200  $\mu\text{m}$ .

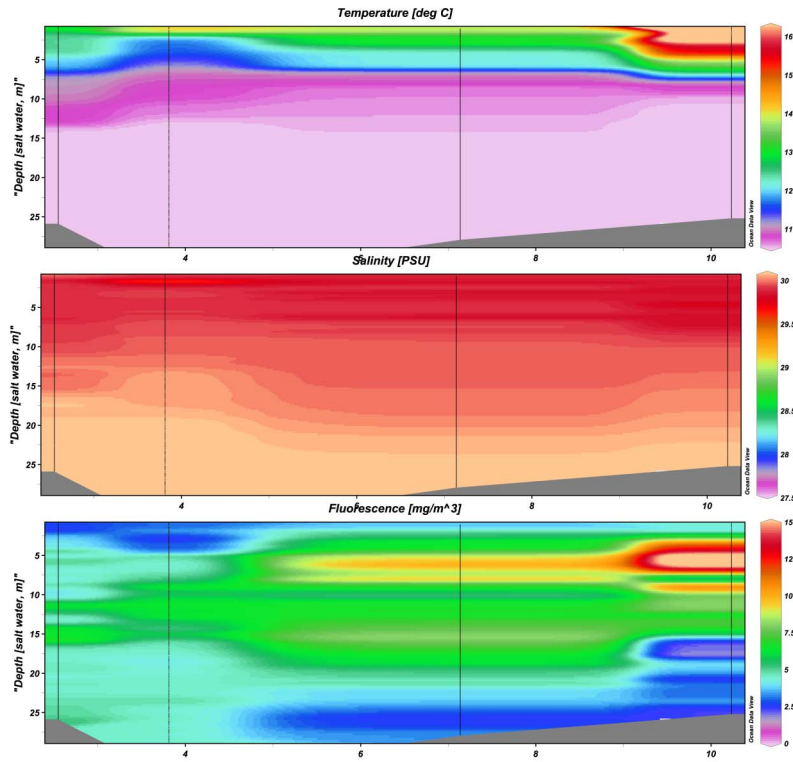
Rate measurements to decipher plankton dynamics were run on every cruise day, except the first week and the last day. There were three different types of rate measurement experiments, as described above: (1) Dilution experiments to measure removal of phytoplankton through predation, (2) Primary productivity measurements under in-situ conditions and (3) Primary productivity under standardized conditions. Care was taken to run these three types of experiments sequentially in conjunction with each other, so that the different methodological approaches could be related to one another and potentially yield redundancies that would allow streamlining of the methodology. In total 7 dilution experiments were run on three cruise days, including experiments with nutrient additions to measure phytoplankton population dynamics as a function of both nutrient availability as well as zooplankton grazing pressure. A total of 24 in-situ primary productivity experiments were conducted on 4 separate days, representing 14 different stations with 2 depths each, and a total of 144 samples. Primary productivity experiments under simulated conditions, in temperature controlled light boxes and at 14 light levels were conducted on 9 cruise days, representing a total of 57 experiments with a total of nearly 800 individual samples. Both types of primary productivity experiments were conducted using variations in size fractionation and time-course experiments to decipher the particular effect these factors have on the resulting measurements.



**Figure 2.** *Interpolated distributions of, from top, temperature ( °C), salinity (PSU) and fluorescence (volts), across the four sampling stations (black vertical lines). The x-axis indicates distance from the southern terminus of the sound northward from left to right. The toxic alga *Heterosigma akashiwo* formed a dense layer within the warmer, fresher layer at the surface of East Sound on July 18<sup>th</sup> 2007. The layer was only 0.5 m thick. A second, less dense layer can be seen at depth below 5 m at station 1, deepening and intensifying towards the north. This layer consisted of mixed phytoplankton species, rather than the almost mono-specific layer at the surface.*

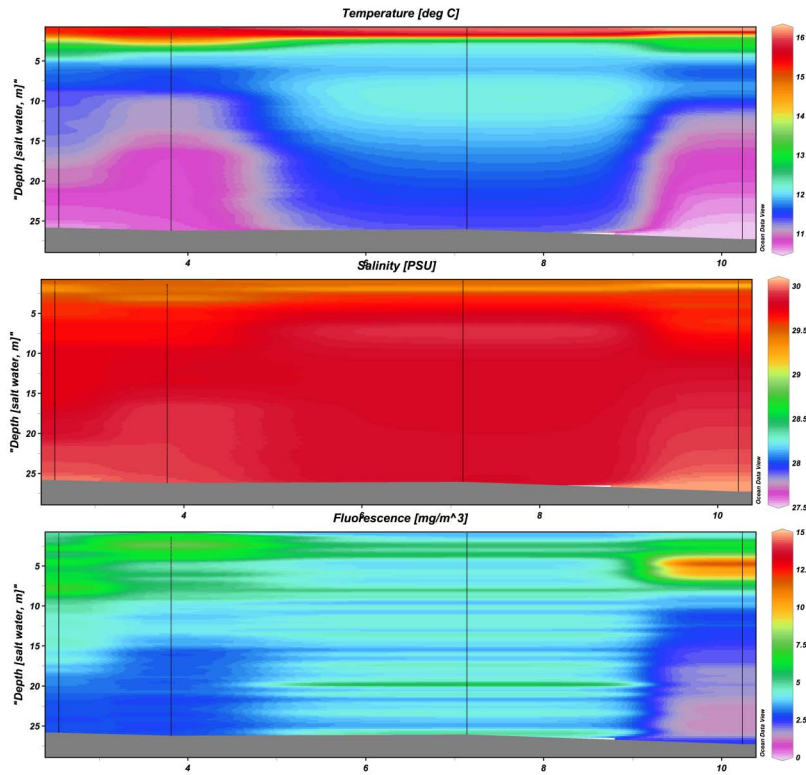
## RESULTS

The intensive sampling effort undertaken to quantify the biological dynamics of plankton rich layers in East Sound, Washington within a physical and chemical context revealed a rich and dynamic picture of the interplay between physical forcing and biological response. Specifically, our results provide multiple examples of patch formation that include in-situ growth as well as organism migrations. Having gathered data with daily and meter-scale resolution provides an exciting opportunity to extrapolate biologically relevant small-scale processes to scales relevant to the optical and acoustical properties of the water column. Since the fieldwork only ended last month, the results presented here have not been subject to the scrutiny of rigorous analysis and need to be considered preliminary.



**Figure 3. A dense layer, dominated by diatoms can be seen extending throughout East Sound. Future analysis will reveal the association between the physical structure and the biological patch. Figure details as in figure 2.**

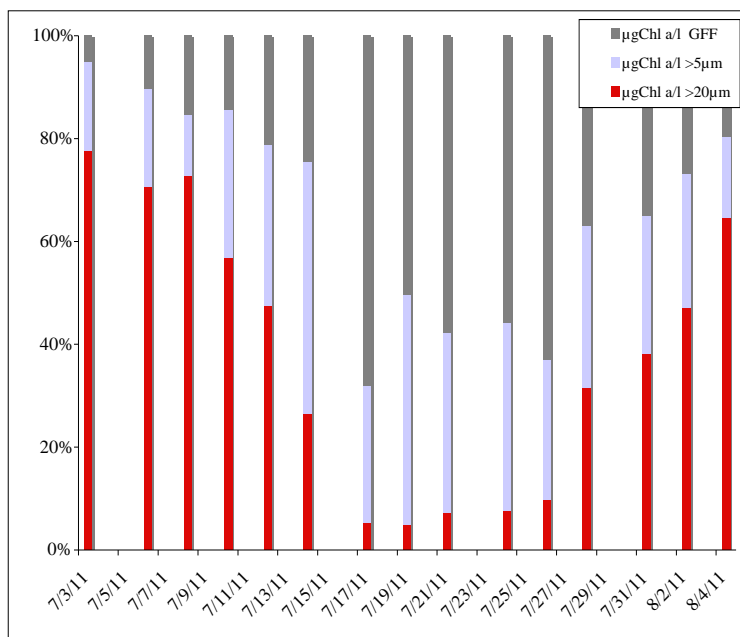
Nonetheless, both during the time on the boat and the subsequent laboratory analysis exiting trends were apparent. We were able to sample in changing conditions over the course of 5 weeks, allowing us to observe the linkages between environmental factors and subsequent biological response, or vice versa. For example, a rain storm, lasting for several days with unseasonably cold temperatures resulted in a freshening of the surface layer. In response, we were able to observe the formation and persistence of a surface layer of the toxic alga *Heterosigma akashiwo* within the fresher surface layer during the week of July 16<sup>th</sup> (Figure 2). This layer persisted for several days and dissipated upon reintroduction of saltier surface water. These observations were collected within the context of biological rate measurements of *H. akashiwo* growth and mortality due to grazing; discussed below. Therefore, our analysis allows us to quantify the effect of each process independently and verify the predictions of population dynamics from laboratory measurements against the field observations collected on subsequent days. These observations confirm our initial suggestion that although physical forcing mechanisms are important for establishing conditions that promote layer presence, it is the biological response that leads to patch formation.



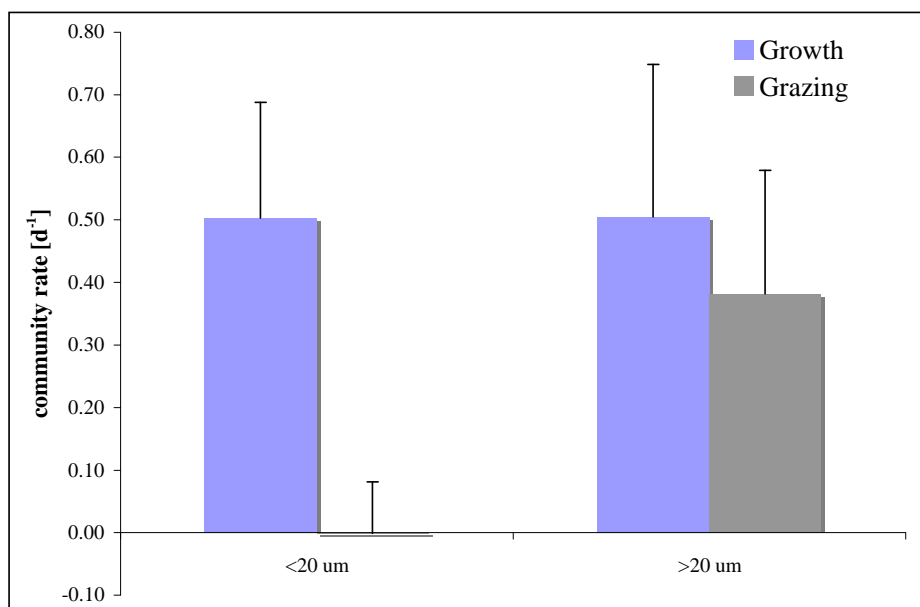
**Figure 4. Appearance of a diatom layer, at about 5 m depth at the north end of East Sound on August 1<sup>st</sup> 2007. Details as in Fig 2.**

A great advantage of having spent an extended time in the field is that we were able to observe different types of patch formation events and the interchange between these events. An example of an extensive diatom layer was observed on July 6<sup>th</sup> 2007 (Figure 3). This layer was dominated by the toxic alga *Pseudo-nitzschia pseudo-delicatissima* (R. Horner, pers. comm.). We subsequently watched this patch disappear and be replaced by a layer of *Heterosigma akashiwo* (Figure 2), which in turn was again replaced by another, less extensive diatom layer (Figure 4). Through this field season, we have made many observations of different phytoplankton patches in a range of conditions. Future analysis of the association between nutrient availability, physical structure and patch characteristics will reveal time and space scales, as well as mechanistic linkages between the environmental conditions and changes in biological structure.

We were able to gather exciting data on the community composition and changes therein, using size fractionated Chl *a* extracts. The precision of the Chl *a* measurements is ~ 10%. Chl *a* concentrations were measured in conjunction with observations of the taxonomy and species composition of the dominant phytoplankton species. Both measures suggest the same trend, whereby the size fractionated Chl *a* concentrations require far less time and effort to gather, and therefore may be an exciting tool in future studies. Over the course of 5 weeks, a clear shift in community composition can be seen in the relative abundance of different size fractions, during times of diatom dominance, early and late in the time series, the >20  $\mu\text{m}$  size fraction dominates Chl *a* concentrations (Figure 5). The emergence and dissipation of the *Heterosigma akashiwo* layer (cell size ~ 10  $\mu\text{m}$ ) can clearly be seen during the middle of the time series. Laboratory tests revealed that *H. akashiwo* sheds its chloroplasts under stress and thus a significant fraction passes the 5 $\mu\text{m}$  filter. Therefore, differences between the two smaller size fractions may not be meaningful.



**Figure 5. Size fractionated Chl *a* composition over 5 weeks. The observed shift between diatoms (>20  $\mu\text{m}$ ) and *Heterosigma akashiwo* (~10  $\mu\text{m}$ ) and back to diatoms can clearly be seen in the time series.**



**Figure 6.** Growth and grazing rates measured on samples from dense *H. akashiwo* layers, station 3 and 4, August 18<sup>th</sup> 2007. Note the grazing rate on the <20μm size fraction is not significantly different from zero.

The proper analysis of the biological rate measurements will take many more weeks. However, the following might serve as an example of the type of result we can anticipate from this analysis. A dilution and growth experiment was conducted on July 18<sup>th</sup>, during the peak of the *Heterosigma akashiwo* layer occurrence (Figure 2). These experiments were analyzed in size fractions to distinguish the dominant components of the plankton, *H. akashiwo* at <20 μm in size, and different diatom species, >20 μm in size. These experiments showed, that whilst both groups were growing at a considerable rate, allowing a theoretical doubling of the population within a day, the smaller size fraction was not grazed upon significantly (grazing rate ~ 0 day<sup>-1</sup>), whereas grazing rate on the larger size fraction, representing diatoms, almost equaled the population growth rate (Figure 6). Based on these experiments, one would predict an increase in the biomass of the smaller size fraction but not the larger size fraction. These predictions were confirmed by our subsequent measurements of increases in the smaller size fraction, but no observable increases in the larger size fraction (Figure 5). Availability of many such experiments may provide a mechanistic insight into the processes regulating population abundance and patch formation.

## IMPACT/APPLICATIONS

As proposed, this work characterized the dynamics of biological patch formation and dissipation with high spatial and temporal resolution. These observations are collected within the context of detailed measurements of physical, chemical and biological parameters, including ecological rate measurements of plankton population dynamics. These concurrent measurements allow identification of the major processes and conditions under which patches form, persist and dissipate. The results of this work provide, to my knowledge, the first estimates of phytoplankton growth and zooplankton grazing rates associated with plankton-rich layers. These results provide quantitative estimates of the

magnitude with which biological processes can mediate changes in the optical and acoustical properties of the water column.

## RELATED PROJECTS

This work builds on previously funded ONR work in East Sound by many investigators and was done in collaboration with Tatiana Rynearson (ONR Award N000140710912) who is investigating how genetic variation affects the development and persistence of plankton layers.

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